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DOI: https://doi.org/10.1007/s00580-013-1869-3

Comp Clin Pathol (2015) 24:117–125
DOI 10.1007/s00580-013-1869-3

ORIGINAL ARTICLE

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Received: 1 October 2013 / Accepted: 9 December 2013 / Published online: 9 January 2014

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Keywords Blood gas analysis · Preanalytical error · Plastic syringe · Sample storage · Storage temperature · Calves

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Introduction

Perinatal mortality is a growing problem in cattle operations (Bleul 2011; Mee et al. 2008). Most neonatal deaths during parturition and within the first 48 h of birth are attributable to hypoxia during parturition (Eigenmann 1981; Szenci et al. 1988). This leads to fetal asphyxia, which is characterised by mixed respiratory and metabolic acidaemia. Correction of the acidaemia through stimulation of respiration and administration of alkalinising medications is imperative for increasing chances of survival (Bleul et al. 2005; Bleul and Bylang 2010; Szenci 1985). However, determination of the severity of acidaemia is difficult by clinical assessment (Götz 2012; Szenci 1982) and is best achieved by venous blood gas analysis values and acid–base measurements (Bleul et al. 2005; Herfen and Bostedt 1999). Unless a suitable handheld machine is available, determination of the latter in food animal practice is usually not feasible in the field, and blood samples must be transported to a laboratory. In contrast to blood samples destined for haematological or biochemical evaluation, which yield reliable results within a sufficient storage period (Bleul et al. 2002; Blincoe and Marble 1985), blood gas variables change quickly depending on storage temperature and may render clinically useless measurements (Piccione et al. 2007). The maximum length of time blood samples can be stored for blood gas analysis without affecting values in cattle is controversial; recommendations vary from 1 h to 1 day (Cingi et al. 2009; Gokce et al. 2004b; Szenci and Besser 1990). The effects of the syringe type and anticoagulant on blood gas values were not investigated in those studies, and the blood samples were collected from healthy cows and calves. In clinical practice, samples for blood gas analysis are usually collected from ill animals that are likely to have abnormal values. Thus, the goals of the present study were to investigate the effects of syringe type, anticoagulant, storage temperature, storage time and severity of acidaemia on the

blood gas and acid–base values of the venous blood of calves with mixed respiratory and metabolic acidaemia. It was hoped that the time frame and storage conditions for ensuring accurate results could be determined and applied to large animal practice.

Materials and methods

Samples

For comparison of the effects of heparinised syringes and commercial blood gas syringes on blood gas and acid–base variables, a total of 240 venous blood samples were collected from 30 newborn calves, which were delivered via Caesarean section (n=22) or vaginally (n=8). A 1.8×16-mm (Vygonüle T, Vygon, Aachen, Germany) indwelling catheter was placed in either the left or right external jugular vein to facilitate blood collection at 40, 60, 120, 240 and 720 min and 24, 48 and 72 h postnatum. The samples were analysed immediately after collection.

For the study of the effect of storage of blood samples for up to 12 h, blood samples from the same 30 calves were used. Jugular blood samples were collected immediately after birth via venipuncture with a needle (1.2×40 mm, Neolus, Terumo, Japan). Of the 30 calves, 18 were retrospectively identified as acidaemic based on a blood pH of <7.2 and the remaining 12 calves as non-acidaemic based on a blood pH of ≥7.2.

Blood samples were collected into two commercial blood gas syringes (Monovette, Sarstedt, Nümbrecht-Rommelsdorf, Germany) and one heparinised syringe (3 mL, Omnifix, Braun-Melsungen, Melsungen, Germany). The Monovette blood gas syringe (Mono) is coated with calcium-balanced lithium heparin in a concentration of 50 IU/mL blood when the syringe is filled to the 2-mL mark. Air bubbles were carefully removed from the Mono before it was sealed with a cap supplied by the manufacturer. The single-use heparinised syringes referred to as HS were used to aspirate a small amount of sterile sodium heparin in a concentration of 5,000 IU/mL (Bichsel, Interlaken, Switzerland), which was then expelled from the syringe barrel by pushing the barrel forward. The amount of heparin that remained in the dead space of the syringe corresponded to the adapter volume of 0.08 mL (Gokce et al. 2004b). Once the syringe was prepared in this manner, it was also filled to the 2-mL mark with venous blood. Air bubbles were removed from the HS before it was appropriately capped with an air-tight rubber cap. The heparin and blood were gently mixed by inverting the syringe several times. Within 5 min of collection, the blood in both syringe types was analysed for pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), base excess (BE), bicarbonate (HCO_3^-) concentration, oxygen saturation (sO_2), total carbon dioxide (TCO_2) and L-lactate concentration using the i-STAT (Abbott, Chicago, USA) system and the corresponding manufacturer's CG4+ (Abbott, Chicago, USA) cartridges to acquire baseline values. The analyser consisted of a handheld machine and single-use cartridges that combined various miniaturised microsensors, a single-point calibration system, fluid channel and waste chamber. The type of syringe (Mono versus HS) containing the venous blood was alternated with each analysis.

For investigation of the effects of storage temperature and storage time on blood gas and acid–base variables, the same samples were processed as follows: One of the two Mono for each sample was stored in the refrigerator at 4 °C and the other at room temperature at 18 °C, and the HS was stored at room temperature. The blood stored in these three syringes underwent blood gas analysis at 20, 40, 60, 120, 180, 360 and 720 min after collection.

Statistical analysis

The StatView software (SAS Institute, Cary, USA) was used for analysis. Venous blood from a healthy calf was collected immediately postnatum in a Mono and analysed 15 times one after the other using the i-STAT analyser to determine intraserial precision, and means and standard errors were calculated for each variable. The range of ±3 standard deviations of the intraserial precision was used as a cutoff for the deviations attributable to analyser variability to differentiate between changes in the variables with respect to time of storage and the inherent imprecision of the i-STAT system (Kraft and Dürr 2005).

Linear regression analysis was used to compare results of Mono and HS, and Bland–Altman difference plots with 95 % limits of agreement (mean difference ±2 SD) were generated (Bland and Altman 1986). In addition, Pearson's coefficient of correlation (r), bias (mean of the difference), intercept and slope with 95 % confidence intervals were calculated. Coefficients of correlation >0.9, between 0.9 and 0.7, smaller than 0.7 and greater than 0.5, and ≤0.5 indicated very strong, strong, moderate and weak correlations, respectively.

Two-factorial ANOVA was used to analyse the effect of storage of the blood on blood gas measurements. Fisher PLSD test was used for comparison of the means of the different storage times and initial value, and for comparison of the means of the two syringe systems at the different storage times. p values≤0.05 were considered significant.

Approval of animal use

The use of animals for this study was approved by the cantonal veterinary office of Zürich (permit number 31/2009).

Results

Effect of syringe type on blood gas and acid–base values

The relationship between the results of samples collected into Mono and HS is shown in Table 1. Regression analysis revealed a significant correlation between the results of all variables for the two syringe types. The pH, BE, HCO_3^-, TCO_2 and L-lactate concentrations were very strongly correlated and had a small bias. The correlations of BE, HCO_3^- and TCO_2 had a small proportional systematic error leading to the underestimation of measurements from HS that increased with increasing values. The pH results also had a proportional systematic error leading to HS values below 7.2 greater and above 7.2 smaller than values from Mono. A similar proportional systematic error also occurred with the variables PCO_2, PO_2 and sO_2 (Fig. 1), which had considerably weaker correlations (Table 1).

Effect of storage time and storage temperature on blood gas and acid–base results

pH

Analysis of variance of the pH profiles as a function of storage temperature revealed a significant difference ($p<0.0001$; Fig. 2). The baseline values differed minimally from each other (mean group, 4 °C, 7.13±0.14; mean group, 18 °C, 7.14± 0.14), and the values during the first 2 h were very similar. The means of the two temperature groups differed significantly after 12 h (4 °C, 7.09±0.13; 18 °C, 6.98±0.15, $p<0.0001$). The means for blood stored at 4 and 18 °C were significantly different from baseline after 180 and 60 min, respectively.

The syringe type had a significant effect on the pH profile ($p<0.0001$); after 12 h in HS, the pH had decreased from 7.13 ±0.15 to 7.01±0.15 and after 12 h in Mono, from 7.14±0.14 to 6.98±0.15. The means for blood stored in HS and Mono were significantly different from baseline after 180 and 120 min, respectively.

Partial pressure of CO_2

Storage temperature had a significant effect on the pCO_2 profiles ($p<0.0001$; Fig. 3). Between blood collection (4 °C, 69.92±11.98 mmHg; 18 °C, 69.58±11.3 mmHg) and 180 min (4 °C, 72.1±10.37 mmHg; 18 °C, 72.63± 11.78 mmHg), the two profiles were all but congruent, after which values for samples stored at 18 °C increased to 90.79±15.39 mmHg, and values for samples stored at 4 °C increased to 75.82±10.78 mmHg by 12 h ($p<0.0001$). The means for blood stored at 4 and 18 °C were significantly different from baseline beginning at 180 and 120 min, respectively.

The syringe type had a significant effect on the pCO_2 profile ($p<0.0001$; Fig. 4). After 3 h of storage, the values from samples stored in Mono had increased at a higher rate than those from samples stored in HS, and the means were significantly different at 6 and 12 h. The means for blood stored in HS and Mono were significantly different from baseline after 180 and 120 min, respectively.

Partial pressure of oxygen (pO_2)

Storage temperature of the blood samples had a significant effect on pO_2 profiles ($p<0.05$). Baseline values were similar (4 °C, 20.93±7.24 mmHg; 18 °C, 21.17±7.23 mmHg), but after 720 min, samples stored at 18 °C had greater pO_2 than those stored at 4 °C (31.73±8.52 mmHg versus 28.55± 13.07 mmHg). The pO_2 in samples stored at 4 °C was significantly greater than baseline from 60 min to the end of the study period.

The type of syringe had a significant effect on the pO_2 profiles ($p<0.01$; Fig. 5). Baseline pO_2 was 4.23 mmHg higher in HS than in Mono, and this difference remained for the first 2 h (all $p<0.05$). The means of blood stored in HS and Mono were significantly different from baseline from 360 to 60 min, respectively.

Table 1 Coefficients of correlation, linear regression with intercept and slope, bias and 95 % limits of agreement of the Bland–Altman analysis between HS and Mono

Variable	Coefficient of correlation (r)	95 % limits of agreement	Intercept with 95 % CI	Slope with 95 % CI	Bias with 95 % CI
pH	0.947	−0.01 to 0.09	0.55 (0.24 to 0.87)	0.92 (0.88 to 0.97)	−0.002 (−0.008 to −0.005)
pCO_2 (mmHg)	0.782	−13.17 to 21.28	26.71 (21.79 to 331.63)	0.58 (0.52 to 0.65)	4.05 (2.93 to 5.17)
pO_2 (mmHg)	0.477	−17.33 to 13.53	17.14 (14.37 to 19.91)	0.40 (0.29 to 0.50)	−1.90 (−2.90 to −0.89)
BE (mmol/L)	0.971	−2.81 to 4.38	−0.936 (−1.28 to −0.60)	0.98 (0.94 to 1.01)	0.79 (0.55 to 1.02)
HCO_3^- (mmol/L)	0.974	−1.45 to 3.487	0.24 (−0.50 to 0.98)	0.95 (0.92 to 0.98)	1.02 (0.86 to 1.18)
sO_2 (%)	0.521	−29.04 to 22.23	18.85 (15.57 to 22.13)	0.45 (0.34 to 0.55)	−3.41 (−5.08 to −1.74)
TCO_2 (mmol/L)	0.968	−1.61 to 3.90	0.64 (−0.25 to 1.53)	0.93 (0.90 to 0.96)	1.15 (0.97 to 1.33)
L-Lactate (mmol/L)	0.988	−1.25 to 1.55	−0.15 (−0.43 to 0.14)	1.00 (0.98 to 1.02)	0.15 (0.05 to 0.24)

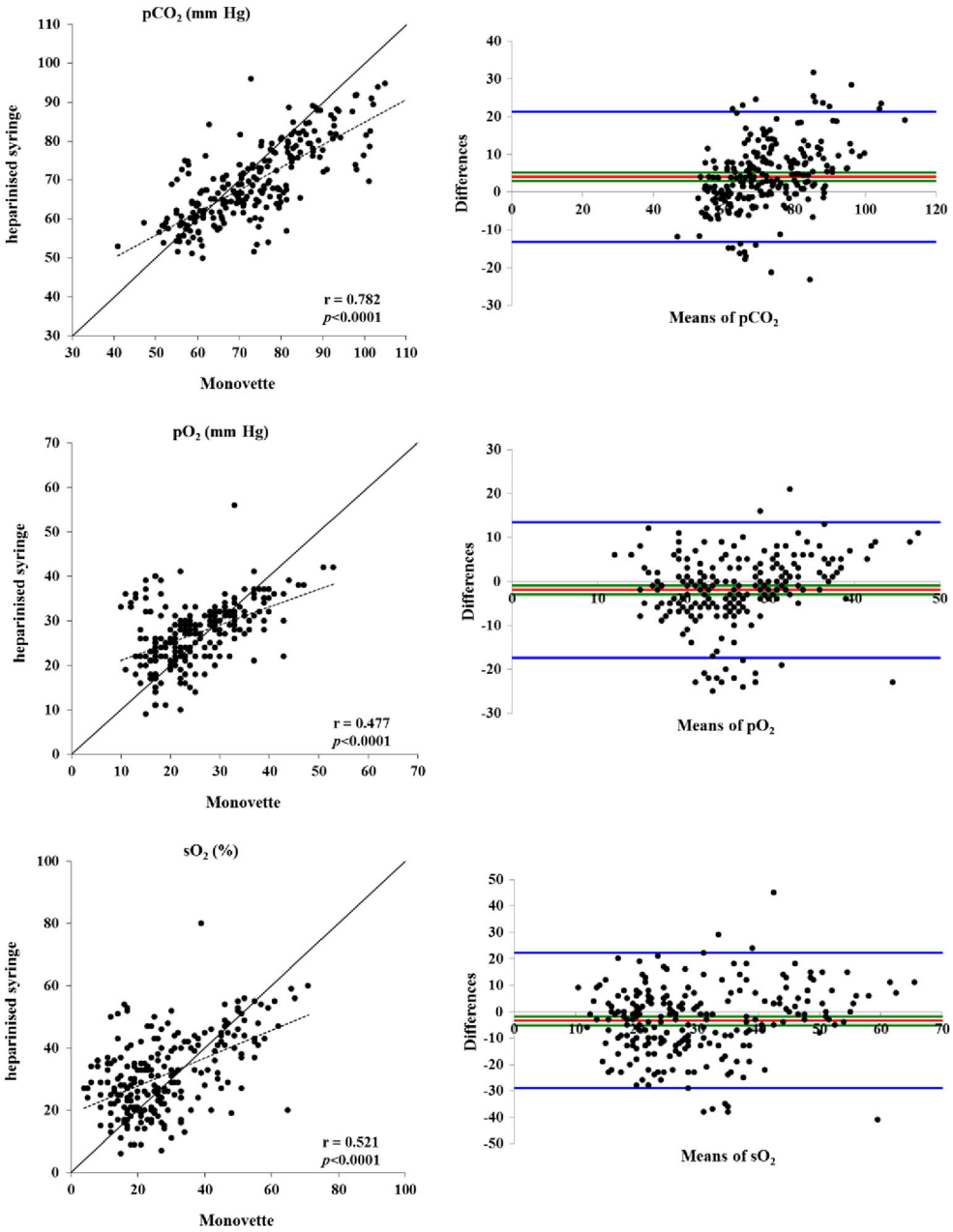

Fig. 1 Regression scatterplots (*left*) and Bland–Altman difference plots (*right*) for comparison of results from the heparinised syringe and Monovette for the variables PCO_2, PO_2 and sO_2. In the regression scatterplots, the *solid line* is the line of identity ($y=x$) and the *dotted line* is the line of best fit. In the Bland–Altman plots, the *red line* shows the bias and the *green lines* mark the 95 % confidence interval (CI 95 %). The *blue lines* are the 95 % limits of agreement

Base excess

Storage temperature had a significant effect on the BE profiles ($p<0.0001$; Fig. 6). The profiles were almost congruent during the first hour, after which time, they started to deviate with greater values in samples at 4 °C and a significant difference of 3.17 mmol/L between the temperature groups at 720 min ($p<0.05$). The means for blood stored at 4 °C starting at 180 min and the means for blood at room temperature starting at 60 min were significantly different from baseline.

The type of syringe had no significant effect on the base excess profiles. The means for blood stored in HS and Mono were significantly different at 20 min and between 60 and 720 min, respectively.

Bicarbonate concentration

Storage temperature had a significant effect on the HCO_3^- profiles ($p<0.01$). After similar baseline values (4 °C, 23.79±5.64 mmol/L; 18 °C, 23.84±5.55 mmol/L), the curves diverged increasingly, but the means of the individual measuring points did not differ. None of the measurements differed from baseline in the samples stored at 4 °C, whereas values from 120 to 720 min differed from baseline values in the samples stored at 18 °C.

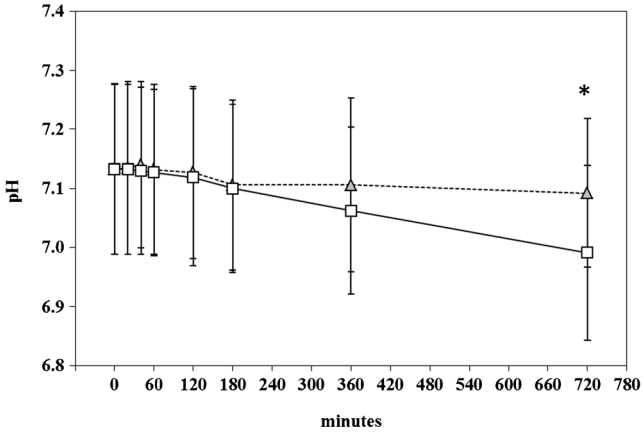

Fig. 2 pH profiles as a function of storage temperature of blood samples. ----- ▲ ---- 4 °C (n=29); —□— 18 °C (n=30). The *asterisk* denotes that means at this time point differ significantly between the groups

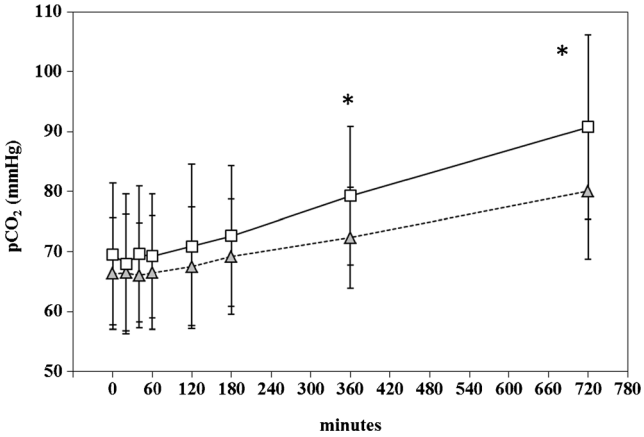

Fig. 4 Profile of pCO_2 as a function of syringe type used for storage of the blood. ----- ▲ ---- heparinised syringe (n=29); —□— Monovette (n=30). The *asterisk* denotes that means at this time point differ significantly between the groups

The type of syringe had no significant effect on the HCO_3^- profiles. All means starting at 120 min were significantly different from baseline in both syringe types.

Oxygen saturation

Storage temperature and syringe type had no effect on the profiles of oxygen saturation. The means of blood stored at 4 and 18 °C differed significantly from baseline after 20 and 120 min, respectively, and of blood stored in HS and Mono after 360 and 120 min, respectively.

L-lactate

Storage temperature had a significant effect on the L-lactate profiles (p<0.0001; Fig. 7). The two curves were parallel for 180 min, after which time, concentrations in blood stored at 18 °C increased to a maximum of 13.96± 3.63 mmol/L at 720 min from the baseline concentration of 10.97±4.66 mmol/L. The means at 720 min differed significantly between the two temperatures. The means of blood at 4 °C did not differ from baseline throughout the observation period, whereas those of samples at 18 °C differed significantly from 180 to 720 min.

The syringe type did not affect the L-lactate profiles. The means of blood stored in HS and Mono differed significantly from baseline from 120 to 720 min and from 180 to 720 min, respectively.

The L-lactate profiles of blood stored in Mono were significantly affected by pH immediately after birth. Calves with a pH<7.2 had an increase in L-lactate of 4.6 %, and calves with a pH≥7.2 had an increase of 29.4 % during the study period.

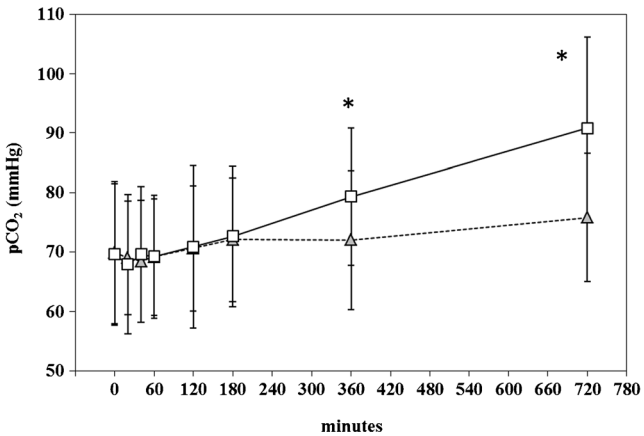

Fig. 3 Profile of pCO_2 as a function of storage temperature of blood samples. ----- ▲ ---- 4 °C (n=29); —□— 18 °C (n=30). The *asterisk* denotes that means at this time point differ significantly between the groups

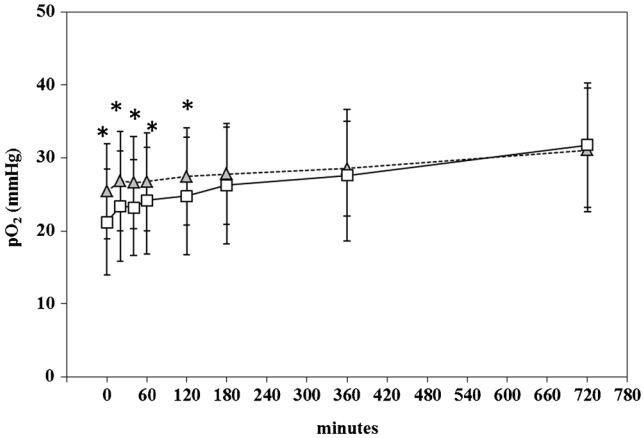

Fig. 5 Profile of pO_2 as a function of syringe type used for storage of the blood. ----- ▲ ---- heparinised syringe (n=28); —□— Monovette (n=30). The *asterisk* denotes that means at this time point differ significantly between the groups

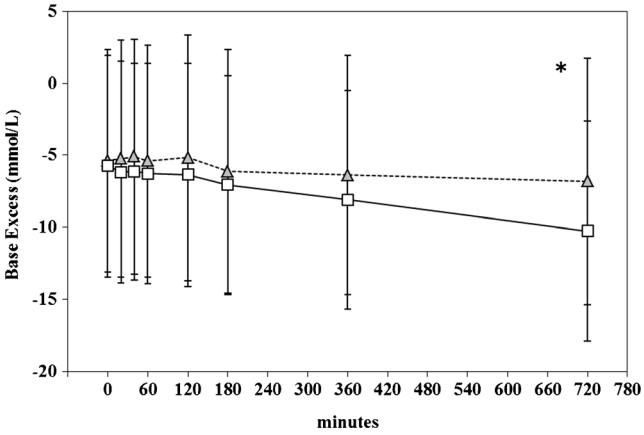

Fig. 6 Profiles of base excess as a function of storage temperature of blood. ----- ▲ ---- 4 °C (n=29); —□— 18 °C (n=30). The *asterisk* denotes that means at this time point differ significantly between the groups

Measurements outside of 3 standard deviations of the inherent imprecision of the analyser attributable to storage temperature and syringe type

The inherent imprecision of the i-STAT system was defined as the range of ±3 SD of the intraserial precision. Therefore, the deviations of means from baseline outside of this range were considered to be attributable to storage conditions. Table 2 shows the range of ±3 SD for blood gas and acid–base variables and the differences between the means for the various storage times and baseline values. The means of pH and pCO_2 of blood stored at 4 °C exceeded the range of ±3 SD of the intraserial precision later than the means of these variables of blood stored at room temperature, whereas the means of pO_2 at 4 °C exceeded the range as early as 20 min and at room temperature at 180 min. The absolute deviations from baseline for base excess and HCO_3^- concentration were within ±3 SD of the intraserial precision 4 °C samples, whereas the ±3 SD range was exceeded at room temperature after 6 and 12 h, respectively. The deviations from baseline for sO_2 and CO_2 concentration were within ±3 SD of the intraserial precision at both temperatures throughout the study period. The same was true for L-lactate at 4 °C, whereas at room temperature, the ±3 SD range was exceeded from 180 min to the end of the study period.

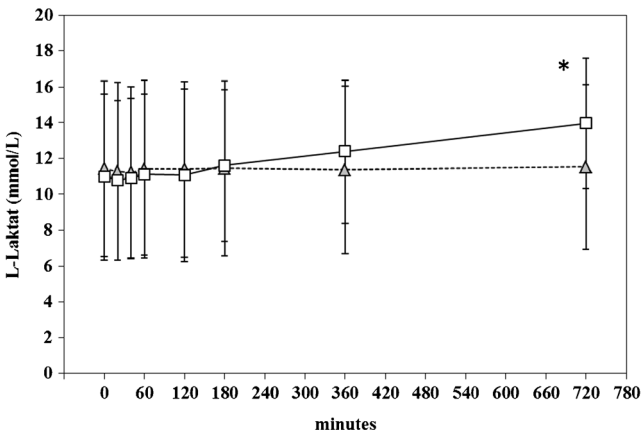

Fig. 7 Profiles of L-lactate concentration as a function of storage temperature. ----- ▲ ---- 4 °C (n=28); —□— 18 °C (n=28). The *asterisk* denotes that means at this time point differ significantly between the groups

In blood stored in HS at room temperature, the L-lactate concentration exceeded the critical limit as early as after 120 min of storage and thus was the first variable to exceed the ±3 SD range of the intraserial precision in HS. As with storage in Mono, the pH of blood stored in HS was outside the ±3 SD range after 3 h. The means of pCO_2, pO_2 and BE were outside of the critical range at 6 h. Oxygen saturation never exceeded the ±3 SD of the intraserial precision, but HCO_3^- and TCO_2 of HS blood did in the last measurement at 12 h.

Discussion

Arterial and venous blood gas analysis and determination of acid–base balance are of central importance in neonatology for both clinicians and researchers; the results are used to determine the type and severity of postnatal disorders and to monitor the efficacy of treatment (Bleul et al. 2005; Bleul et al. 2008; Herfen and Bostedt 1999; Szenci 1985). Reliable determination of these values requires a suitable analyser as well as an appropriate preanalytical protocol for blood collection, storage, transportation and sample preparation. The preanalytical protocol plays an important role in the accuracy of the results (Burnett et al. 1995). In bovine practice, there are at least two factors in the preanalytical protocol that usually differ from protocols used in small animal practice and human medicine; immediate transport of the blood for analysis is seldom possible, and the use of expensive supplies is not feasible. Blood gas syringes are the principal supply item (Kume et al. 2012; Muller-Plathe and Heyduck 1992; Picandet et al. 2007). Glass syringes are considered the gold standard for blood gas analysis because in contrast to plastic syringes, they are all but impermeable to gas (Muller-Plathe and Heyduck 1992; Picandet et al. 2007). However, glass syringes are not commonly used anymore because they are breakable, expensive and may increase the risk of infection when used more than once. The plastic syringes commonly used today are permeable to gas, which can alter blood gas results. The permeability depends on the type of plastic; therefore, most commercially available blood gas syringes are made of polypropylene because this material is reported to be less permeable to gas than other plastics (Picandet et al. 2007). Although the Mono and HS used in this study were made of polypropylene, the permeability of the syringe seemed to have a marked effect on blood gas variables of

Table 2 Range of ±3 standard deviations of the inherent imprecision of the analyser and deviation of the means from baseline in blood stored in Monovette blood gas syringes (Mono) at 4 °C and 18 °C and in heparinised syringes (HS) at 18 °C

	±3 SD	Storage condition	20 min	40 min	60 min	120 min	180 min	360 min	720 min
pH	±0.03	Mono 4 °C	0.003	0.008	0.000	−0.005	−0.026	−0.026	−0.040[a]
		Mono 18 °C	0.006	−0.001	−0.005	−0.01	−0.034[a]	−0.079[a]	−0.157[a]
		HS 18 °C	−0.005	−0.008	−0.012	−0.022	−0.034[a]	−0.063[a]	−0.115[a]
pCO_2 (mmHg)	±5.58	Mono 4 °C	−0.941	−1.500	−0.752	0.686	2.176	2.286	5.897[a]
		Mono 18 °C	−1.647	0.026	−0.344	1.286	3.053	9.733[a]	21.216[a]
		HS 18 °C	0.207	0.353	0.647	1.464	2.888	5.631[a]	12.195[a]
pO_2 (mmHg)	±3.57	Mono 4 °C	3.793[a]	4.172[a]	5.310[a]	5.517[a]	4.586[a]	4.897[a]	7.621[a]
		Mono 18 °C	2.200	2.000	2.966	3.566	5.066[a]	6.433[a]	10.566[a]
		HS 18 °C	0.649	2.382	0.933	1.514	1.874	4.495[a]	6.691[a]
BE (mmol/L)	±2.22	4 Mono 4 °C	0.138	0.276	−0.276	0.207	−0.759	−1.000	−1.449
		Mono 18 °C	−0.400	−0.366	−0.566	−0.500	−1.500	−2.600[a]	−4.933[a]
		HS 18 °C	−0.391	−0.972	−0.533	−0.919	−1.347	−2.293[a]	−4.305[a]
HCO_3^- (mmol/L)	±1.71	Mono 4 °C	0.055	0.100	−0.017	0.257	−0.332	−0.238	−0.165
		Mono 18 °C	−0.240	−0.103	−0.300	−0.160	−0.747	−1.080	−2.057[a]
		HS 18 °C	−0.226	−0.248	−0.364	−0.605	−0.790	−1.284	−2.543[a]
sO_2 (%)	±10.56	Mono 4 °C	6.483	7.621	9.000	9.069	6.241	7.069	10.310
		Mono 18 °C	4.467	3.967	4.834	6.200	7.000	7.234	10.234
		HS 18 °C	0.860	2.070	1.493	1.881	2.072	3.188	3.677
TCO_2 (mmol/L)	±1.77	Mono 4 °C	0.035	0.104	0.035	0.345	−0.207	−0.345	0.000
		Mono 18 °C	−0.233	−0.033	−0.267	−0.233	−0.633	−0.667	−1.333
		HS 18 °C	−0.281	−0.309	−0.364	−0.603	−0.712	−1.110	−2.079[a]
L-lactate (mmol/L)	±0.51	Mono 4 °C	−0.139	−0.188	−0.006	−0.014	0.029	−0.050	0.114
		Mono 18 °C	−0.168	−0.073	0.158	0.112	0.618[a]	1.421[a]	2.988[a]
		HS 18 °C	0.097	0.179	0.280	0.599[a]	0.753[a]	1.539[a]	3.026[a]

[a] Difference between mean and baseline is outside ±3 SD of the serial imprecision of the analyser

stored blood samples, and blood gas values differed between the two. The pCO_2 of blood stored in the Mono increased more sharply and faster than that of blood stored in the HS. Two factors play a role in pCO_2 of stored blood samples: Aerobic metabolism in leukocytes, reticulocytes and thrombocytes produces CO_2, the amount of which varies with the concentration of the various cell types (Beaulieu et al. 1999); and CO_2 diffuses from an area of high concentration (blood sample) to a region of low concentration (surrounding air). Assuming that samples in both syringes have the same concentration and composition of cells, it appears that equilibration of pCO_2 between the blood and atmosphere does not occur as readily in the Mono compared with the HS because the pCO_2 increased at a higher rate in the former. In contrast, the pO_2 increased rapidly within the first 20 min and only slightly thereafter. Compared with baseline pressure, significant differences were first seen after 360 min in the HS and after 60 min in the Mono. The loss of oxygen through aerobic glycolysis seemed to have been more than counterbalanced by the influx of oxygen attributable to the gradient between the atmosphere and the blood sample (Mahoney et al. 1991; Picandet et al. 2007). However, there was a significantly higher pO_2 in HS samples than in Mono samples at the first measurement, which was within 5 min of collection. It is plausible that this difference was due to the entry of oxygen through the syringe wall, but failure to remove all air bubbles from the sample seems more likely. It was often difficult to remove all the small air bubbles located at the transition from the syringe barrel to the needle adapter in the HS.

In addition to the inherent analyser imprecision, the previously discussed factors may have been responsible for the correlations between the Mono and HS for pCO_2 (good) and pO_2 (moderate). The correlation for sO_2 between the two syringe types was also only moderate; however, this was probably due to the moderate correlation for pO_2 because sO_2 was calculated using the pO_2. On the other hand, correlations were very strong for acid–base variables, and measurements obtained from HS blood were as accurate as those from Mono. However, strong correlations are not a measure of the accuracy of results; for instance, the measurements of pH and HCO_3^- concentration in HS and Mono samples immediately after collection differed significantly, the means of BE and L-lactate concentration measured in HS samples differed from baseline after a shorter storage time than those measured in

Mono samples, and the means of the blood gas variables pCO_2, pO_2 and sO_2 deviated from baseline earlier in samples stored in Mono than those in HS. A possible reason for these differences is the physical state of the heparin used; liquid heparin can affect the measurements twofold: The anticoagulant causes a certain degree of blood thinning, and it reduces the pH because it is an acid with a pH of 6.4 (Higgins 2007). Provided that the concentration of liquid heparin does not exceed 200 IU/mL of blood and at least a 20-fold volume of blood is added to the heparin (Burnett et al. 1995; Siggaard Andersen 1961), a dilution artifact does not occur with small amounts of liquid heparin (Hutchison et al. 1983; Siggaard Andersen 1961). The amount of approximately 0.08 mL of liquid heparin used in the present study resulted in 200 IU/mL blood, and the 2-mL amount of blood collected corresponded to a 25-fold heparin volume. Furthermore, heparin did not decrease the pH; although the syringe type had a significant effect on the pH profiles, the profiles were nearly parallel and the decrease in pH was numerically greater in blood stored in Mono (0.15 units) than in blood stored in HS (0.13 units). Other studies also reported minimal effects of the pH of heparin on the pH of blood (Hopper et al. 2005; Hutchison et al. 1983), and this was explained by the high buffering capacity of blood. However, in our study, the profiles of HCO_3^- concentration and BE were not affected by the syringe type although BE was slightly lower than baseline after 20 min of storage in HS, which could be due to the liquid heparin. Deviations of measurements from baseline should be interpreted with caution. The small decrease in BE of 0.4 mmol/L after 20 min of storage could also be attributed to variability caused by analyser imprecision. For differentiation of variation attributable to this imprecision and true effects on variables caused by storage conditions, ±3 SD of the intraserial precision of the analyser was used as a cutoff. Using this threshold, there were no effects of syringe type during the first 60 min. After 2 h, L-lactate concentration of blood stored in HS was the only variable with a difference outside the ±3 SD range. Because pO_2 tended to increase in the stored blood samples, anaerobic glycolysis in leukocytes was considered unlikely. Anaerobic glycolysis is the principal process for generating energy in erythrocytes because of a lack of enzymes required for aerobic glycolysis. This results in an increase in the concentrations of L-lactate and pyruvate in the sample, which also contributes to a fall in pH (Greenbaum et al. 1967). This led to a storage-related deviation in pH in HS and in Mono samples from 180 min. With the exception of L-lactate concentration, which should be measured within 1 and 2 h of blood collection, respectively, all blood gas and acid–base variables can be measured in samples kept in HS at room temperature for up to 3 h without generating clinically relevant changes in the results. Because of clinically relevant changes in pH and pO_2, blood should not be kept in Mono for more than 2 h.

The profile of L-lactate was the only one affected by the blood pH at the time of collection. It is possible that calves with a blood pH≥7.2 had higher concentrations of glucose available for anaerobic glycolysis than calves with a blood pH <7.2; lactate had been already generated by the latter group of calves during parturition.

The chilling of blood samples intended for blood gas analysis is widely recommended because of changes with time caused by temperature-dependent enzymes, which function optimally at body temperature (Foster and Terry 1967; Liss and Payne 1993). Chilling can slow down enzymatic processes in anaerobic and aerobic glycolysis, which should result in stabilisation of the blood constituents. This was confirmed in our study and was in agreement with other observations (Gokce et al. 2004a; Poulsen and Surynek 1977; Szenci and Besser 1990); the decrease in pH of blood stored at room temperature was more pronounced than in chilled blood. This can be explained by increased L-lactate and CO_2 production at room temperature, which became particularly noticeable during the third and fourth hours of storage. As a result of this, HCO_3^- concentration and BE also decreased.

While these changes took hours to occur, the mean pO_2 differed from baseline as early as 1 h after storage at both temperatures. However, the mean pO_2 exceeded ±3 SD after 20 min of storage at 4 °C and after 180 min at 18 °C. There are two possible explanations for these observations: At room temperature, the influx of O_2 is counterbalanced by increased O_2 consumption to a higher degree than at 4 °C, which is the temperature at which oxygen-dependent processes are reduced. Additionally, chilling of blood increases the solubility of oxygen and the oxygen affinity of haemoglobin, both of which result in decreased pO_2 in Mono and thus a higher O_2 gradient from the surrounding air to the syringe (Mahoney et al. 1991).

The results of our study indicate that storage of blood in plastic syringes intended for blood gas and acid–base studies should be avoided because of rapid diffusive exchange of gases across the syringe wall. Immediate analysis produces reliable results, but when this is not possible, chilled glass syringes should be used because diffusive gas exchange across glass is minimal (Wiwanitkit 2006). However, glass syringes and commercial plastic blood gas syringes are expensive, and normal syringes are readily available. The Mono used in our study was seven times more expensive than the HS, and storage of the blood in both syringe types at room temperature for up to 3 h did not result in clinically relevant changes in the measurements. As recommended in a study on horses (Picandet et al. 2007), the blood should not be stored on ice. An exception is the determination of L-lactate, for which chilled blood is more suitable when the measurement is delayed.

Conflict of interest The authors declare no conflict of interest.

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